<Division of Environmental Chemistry> Molecular Microbial Science

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Visitors
Prof JHEE, Kwang-Hwan — Department of Applied Chemistry, Kumoh National Institute of Technology, Korea, 27 February 2008–17 February 2009
Dr ABE, Fumiyoshi — Japan Agency for Marine-Earth Science and Technology, 20 October 2009

Scope of Research

Structures and functions of biocatalysts, in particular, pyridoxal enzymes and enzymes acting on xenobiotic compounds, are studied to elucidate the dynamic aspects of the fine mechanism for their catalysis in the light of recent advances in gene technology, protein engineering and crystallography. In addition, the metabolism and biofunction of sulfur, selenium, and some other trace elements are investigated. Development and application of new biomolecular functions of microorganisms are also studied to open the door to new fields of biotechnology. For example, cold-adaptation mechanism and applications of psychrotrophic bacteria are under investigation.

Research Activities (Year 2009)

Presentations

Cold-adaptation Mechanism of An Antarctic Psychrotrophic Bacterium, *Shewanella livingstonensis* Ac10, Kurihara T, Xth SCAR International Biology Symposium, 27 July 2009.

Eicosapentaenoic Acid Plays An Important Role in Assembly of Cell Division Machinery of An Antarctic Sea Bacterium, *Shewanella livingstonensis* Ac10, Kawamoto J, Xth SCAR International Biology Symposium, 27 July 2009.

Phospholipids Involved in Cold Adaptation of An Antarctic Psychrotrophic Bacterium, *Shewanella livingstonensis* Ac10, Kurihara T, NAIST Global COE International Symposium 2009 Environmental Adaptation, 13 November 2009.

Grants

Esaki N, Structure-Function Analysis of Selenium-specific Chemical Conversion System and Co-translational Insertion of Selenium into Protein, Grant-in-Aid for Scientific Research (B), 1 April 2007–31 March 2009.

Kurihara T, Exploration of Novel Cold-adapted Microorganisms to Develop a System for the Production of Useful Compounds at Low Temperatures, Grant-in-Aid for Scientific Research (B), 1 April 2007–31 March 2009.

Kurihara T, Analysis of the Molecular Basis for Cold Adaptation of Psychrotrophic Bacteria, Grant-in-Aid for Scientific Research (B), 1 April 2008–31 March 2011.

Occurrence of a New Enzyme That Catalyzes the Degradation of Unsaturated Organohalogen Compounds

Enzymes catalyzing the conversion of organohalogen compounds are useful in chemical industry and environmental technology. A soil bacterium, *Pseudomonas* sp. YL, inducibly produced a protein named CAA67_YL when the cells were grown on 2-chloroacrylate (2-CAA). The *caa67_YL* gene encoded a protein of 547 amino acid residues, which showed weak sequence similarity to various flavoenzymes. We found that 2-CAA is converted into pyruvate when the reaction was carried out with purified CAA67_YL in the presence of FAD and a reducing agent under anaerobic condition, indicating that $FADH_2$ is required for the reaction. When the reaction was carried out in the presence of $H_2{}^{18}O$, [^{18}O]-pyruvate was produced. This result implies that CAA67_YL catalyzes the hydration of 2-CAA to form 2-chloro-2-hydroxypropionate, which is chemically unstable and probably spontaneously dechlorinated to form pyruvate. 2-Bromoacrylate, but not other 2-CAA analogs such as acrylate and methacrylate, served as the substrate of CAA67_YL. Thus, we named this new enzyme 2-haloacrylate hydratase. The enzyme is very unusual in that it requires the reduced form of FAD for hydration, which involves no net change in redox state of the coenzyme or substrate.

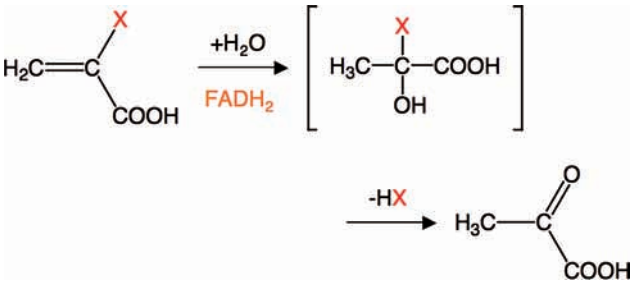

Figure 1. Reaction catalyzed by 2-haloacyrlate hydratase.

Physiological Roles of Eicosapentaenoic Acid-containing Phospholipids in Cold Adaptation of an Antarctic Bacterium, *Shewanella livingstonensis* Ac10

Various bacteria favor cold environments including Polar Regions, glacier, and deep sea. Some cold-adapted bacteria produce polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid, as a component of their membrane phospholipids, suggesting that PUFAs play important physiological roles in their cold adaptation. *Shewanella livingstonensis* Ac10, a cold-adapted Gram-negative bacterium isolated from Antarctic seawater, grows at range of temperatures from 4˚C to 25˚C and produces EPA at 4˚C as a component of membrane phospholipids. The mutant lacking EPA showed significant growth retardation and became filamentous at 4˚C but not at 18˚C, indicating that the mutant has a defect in cell division at low temperatures. Interestingly, the EPA-less mutant developed multiple-intracellular membranes in its cell, suggesting that the deletion of EPA affects the physiological function of proteins involved in membrane biogenesis at low temperatures. FtsEX is supposed to be an ABC transporter composed of ATP binding domain (FtsE) and membrane-spanning domain (FtsX) and plays a role in the membrane-phospholipid transport at cell division site in Gram-negative bacteria. FtsE was localized to the cell membrane in the parent strain, but not in the EPA-less mutant. These results suggested that EPA supports the function and membrane localization of proteins related to cell division.

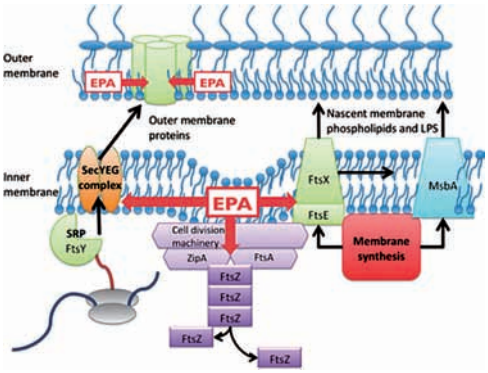

Figure 2. Schematic illustration of physiological roles of EPA in the cold adaptation of *Shewanella livingstonensis* Ac10.

Kawamoto J, Development of a System for the Bioremediation of Rare Metal Pollution and the Rare Metal Recovery Using Novel Metal-Metabolizing Bacteria, Grant-in-Aid for Scientific Research (B), 1 April 2009–31 March 2012.

Award

Kawamoto J, Poster Presentation Award, Physiological Role of Eicosapentaenoic Acid-containing Phospholipids in Refolding of a Cold-inducible Porin, Japan Society of Extremophiles, 28 October 2009.